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Bcl-2 Rescues T Lymphopoiesis, but Not B or NK Cell Development, in Common γ Chain–Deficient Mice

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Summary

The common cytokine receptor γ chain (γ_c) is an indispensable subunit for the formation of lymphoid-related cytokine receptors, including IL-7 and IL-15 receptors, that mediate nonredundant or critical signals for the differentiation of T and B cells and natural killer (NK) cells, respectively. We introduced the *bcl-2* transgene driven by Eμ or H-2K promoters into γ_c-deficient mice that lack all three lymphoid subclasses. The forced expression of Bcl-2 restored all stages of T lymphopoiesis, but not B or NK cell development, indicating that a primary function of γ_c-mediated signals in the T lineage might be to maintain cell survival. Therefore, the development of T, B, and NK cells may be influenced by distinct intracytoplasmic signaling cascades that are activated by coupling of γ_c-related receptors.

Introduction

The common cytokine receptor γ chain (γ_c) is a shared subunit of receptors for interleukin-2 (IL-2) (Takeshita et al., 1992), IL-4 (Kondo et al., 1993; Russell et al., 1993), IL-7 (Noguchi et al., 1993a; Kondo et al., 1994), IL-9 (Kimura et al., 1995), and IL-15 (Giri et al., 1994). A mutation in the γ_c gene can result in a nonfunctional receptor and cause X-linked severe combined immunodeficiency (X-SCID) in humans (Noguchi et al., 1993b; Puck et al., 1993). X-SCID is characterized by a severe impairment of T and natural killer (NK) cell development. Genetic ablation of the γ_c chain in mice causes a severe reduction in the numbers of B cells as well as T and NK cells (Cao et al., 1995; DiSanto et al., 1995; Ohbo et al., 1996). These data indicate that the γ_c chain is an indispensable molecule for the development of all three classes of lymphocytes.

Among cytokine receptors in which the γ_c chain is used, the IL-7 receptor (IL-7R) transmits the nonredundant signals for maintaining T and B lymphopoiesis. Both IL-7 knockout (KO) and IL-7Rα KO mice have a reduction in the number of T and B cell precursors (Peschon et al., 1994; von Freeden-Jeffry et al., 1995), a phenotype that resembles that of γ_c KO mice (Cao et al., 1995; DiSanto et al., 1995; Ohbo et al., 1996). On the other hand, the development of NK cells is not affected in IL-7Rα KO mice (He and Malek, 1996; Maki et al., 1996). NK cell development is inhibited in mice deficient for IL-2Rβ (Suzuki et al., 1997), which is an indispensable subunit of the functional IL-2R and IL-15R complexes (Giri et al., 1994). Since IL-2 KO mice have normal NK cells, it could be that the impaired development of NK cells in γ_c KO mice is due to a lack of functional IL-15R (Kündig et al., 1993).

γ_c plays a role in the maintenance of cell survival, at least in the T cell lineage, through interactions between cytokines and cytokine receptors that require the γ_c subunit. In peripheral T cells from γ_c KO mice, low levels of endogenous Bcl-2 expression correlate with impaired in vitro survival (Nakajima et al., 1997). Recently we reported that IL-7 maintains the expression of endogenous Bcl-2 in the T lineage cells and that the forced expression of Bcl-2 in the T lineage could significantly restore positive selection of T cells in IL-7Rα KO mice (Akashi et al., 1997), although the restoration of T lymphopoiesis was not complete. Since Bcl-2 has been shown to maintain cell survival (Cory, 1995; Yang and Korsmeyer, 1996), it is strongly suggested that the γ_c-mediated defect in T lymphopoiesis may be due to a lack of survival signals from IL-7R. However, it is unknown whether Bcl-2 can substitute for IL-7R–mediated signals in early T cell development, because the Eμ-*bcl-2*-25 transgene failed to be expressed in the earliest thymic precursors such as $CD3^-CD25^-c\text{-}Kit^+$ cells (Akashi et al., 1997). In addition, the restoration of T lymphopoiesis in *bcl-2* transgenic IL-7Rα KO mice could be due to substitution for signals mediated by receptors other than IL-7R, since the IL-7Rα chain has been shown to be shared at least by one other cytokine receptor, thymic stromal cell–derived lymphopoietin receptor (Friend et al., 1994).

To clarify the role of survival signals mediated by γ_c-related cytokines in all stages of lymphopoiesis, we crossed γ_c KO mice with Eμ-*bcl-2*-25 transgenic mice and H2K-*bcl-2* transgenic mice. In the H2K-*bcl-2* transgenic mice, the human *bcl-2* transgene was driven by a major histocompatibility (MHC) class I promoter and a Moloney long terminal repeat, and therefore Bcl-2 is expressed in virtually all developmental stages of hematopoietic cells (J. D. and I. L. W., unpublished data). The enforced expression of Bcl-2 in γ_c KO mice throughout each stage of lymphopoiesis resulted in the restoration of T lymphopoiesis, but neither B nor NK cells were rescued. Signals mediated by the γ_c chain other than survival signals might be indispensable for the development of B and NK cells.

Results

Enforced Expression of Bcl-2 Restores Peripheral T Cells but Not B Cells in γ_c-Deficient Mice

We have found that human Bcl-2 is expressed in all hematopoietic cells, including hematopoietic stem cells in H2K-*bcl-2* transgenic mice (J. D. and I. L. W, unpublished data). Figure 1 (top row) shows human Bcl-2 expression in blood cells. There is no significant difference

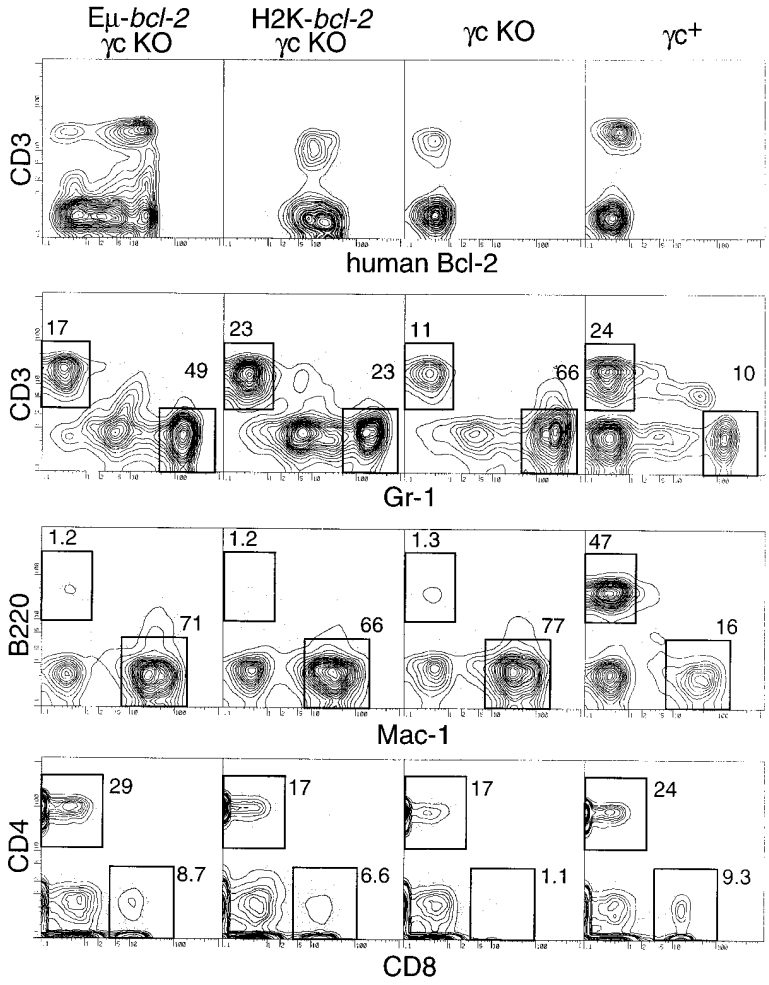

Figure 1. Flow Cytometric Analysis of Nucleated Blood Cells in Each Mouse Strain

(Top row) Expression of the transgene-derived human Bcl-2 protein. The Eμ-*bcl-2* transgene was expressed predominantly in T cells (~85% of T cells), whereas the H2K-*bcl-2* transgene was expressed in virtually all nucleated cells in blood.

(Bottom three rows) CD3/Gr-1, B220/Mac-1, and CD4/CD8 profiles in nucleated blood cells. Numbers accompanying inset boxes indicate the percentage of each population in nucleated blood cells.

in expression levels of human Bcl-2 protein in T cells between Eμ-*bcl-2* and H2K-*bcl-2* transgenic mice. However, the Eμ-*bcl-2* transgene was expressed in approximately 85% of $CD3^+$ T cells, while the H2K-*bcl-2* transgene was expressed in virtually all blood leukocytes. Figures 1 and 2 show the results from an analysis of peripheral blood from each mouse line. Consistent with previous reports, the γ_c KO mice showed a significant reduction in the numbers of $CD3^+$ T cells and $B220^+$ B cells (Cao et al., 1995; DiSanto et al., 1995; Ohbo et al., 1996), whereas the numbers of $Gr\text{-}1^+$ granulocytes remained normal. The ratios of $CD4^+CD3^+$ cells to $CD8^+CD3^+$ cells were widely variable, but were higher than the ratios found in wild type (Figures 1 and 2B), as reported previously (Nakajima et al., 1997).

Introduction of the *bcl-2* transgenes significantly restored the T cell numbers in the blood of γ_c KO mice. In Eμ-*bcl-2* γ_c KO and H2K-*bcl-2* γ_c KO mice, the number of $CD3^+$ cells increased approximately 3-fold (to 20% of γ_c^+ wild-type levels) and approximately 10-fold (to 50% of γ_c^+ wild-type levels), respectively (Figure 2A). The ratio of $CD4^+CD3^+$ cells to $CD8^+CD3^+$ cells in both *bcl-2* transgenic γ_c KO mouse strains was also nearly normal (Figures 1 and 2B). There was no Vβ-specific restoration of peripheral CD4 or CD8 cells in either Eμ-*bcl-2* γ_c KO and H2K-*bcl-2* γ_c KO mice (data not shown).

Although the introduction of either of the two *bcl-2* transgenes restored T lymphopoiesis, it did not rescue the development of B cells in either line (Figure 2A): the absolute number of blood $B220^+$ cells in γ_c KO mice was 0.64% of the number seen in γ_c^+ mice. In Eμ-*bcl-2* γ_c KO mice and H2K-*bcl-2* γ_c KO mice, the numbers of $B220^+$ cells in blood were 1.1% and 1.6% of γ_c^+ wild-type levels. Although the differences between B cell numbers in γ_c KO mice and γ_c KO mice with *bcl-2* transgenes were small, the differences were statistically significant by the Mann-Whitney and Wilcoxon nonparametric tests (Figure 2A).

Enforced Expression of Bcl-2 Restores T Cell Maturation in the Thymus of γ_c-Deficient Mice

We have reported that the enforced expression of an Eμ-*bcl-2* transgene could significantly rescue the positive selection process in IL-7Rα KO mice (Akashi et al., 1997). Similar to the results obtained with the IL-7Rα KO mice, we found that both the absolute number of thymocytes and the number of $CD69^+$ thymocytes that had recently received positive selection were significantly increased in Eμ-*bcl-2* γ_c KO and H2K-*bcl-2* γ_c KO mice (Table 1). The ratio of $CD4^+CD8^-$ to $CD4^-CD8^+$ single-positive thymocytes in γ_c KO mice was apparently higher than in normal mice, as reported previously, but both *bcl-2*

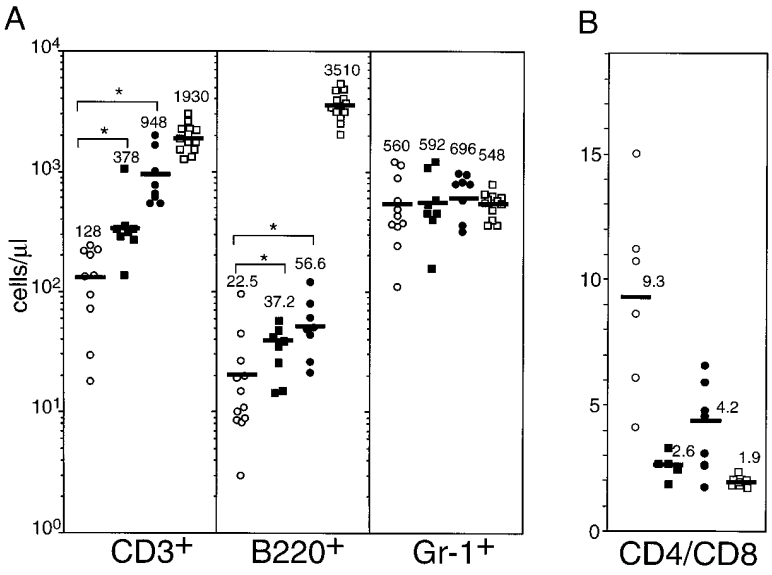

Figure 2. Enforced Expression of Bcl-2 Restores Mature T Cells and Normalizes the Ratio of $CD4^+$ to $CD8^+$ T Cells in γ_c KO Mice

(A) Absolute numbers (cells per microliter) of blood $CD3^+$, $B220^+$ and $Gr\text{-}1^+$ cells. Asterisk, $p < 0.05$ by the Mann-Whitney and Wilcoxon nonparametric tests.

(B) The ratio of $CD3^+CD4^+$ to $CD3^+CD8^+$ single-positive cells in each mouse strain.

Open circles, γ_c KO mice; filled squares, Eμ-*bcl-2* γ_c KO mice; filled circles, H2K-*bcl-2* γ_c KO mice; open squares, control γ_c^+ mice. Mean values are indicated by the bars and adjacent numbers.

transgenes restored the normal ratio (Figure 3A). The restoration of developing thymocytes was more significant in the H2K-*bcl-2* γ_c KO mice. This might be explained by the expression of the H2K-*bcl-2* transgene in virtually all thymocytes (data not shown), whereas the Eμ-*bcl-2* transgene is not expressed in early thymic precursors, including the earliest $CD3^-CD25^-c\text{-}Kit^+$ thymic precursors (Akashi et al., 1997).

Figure 3B shows the c-Kit and CD25 profiles of the $CD3^-$ thymic population in each mouse strain. The earliest thymic precursors that have the potential to develop into B and NK cells as well as T cells (Wu et al., 1991; Matsuzaki et al., 1993) are contained in the $CD3^-CD25^-c\text{-}Kit^{hi}$ fraction (fraction I). The $CD3^-CD25^+c\text{-}Kit^{lo\text{-}hi}$ cells (fractions II and III) have already formed pre-T cell receptor complexes (Saint-Ruf et al., 1994), and their proliferation is synergistically supported by IL-7 and steel factor (Slf) (Godfrey et al., 1993). As shown in Table 1, the absolute numbers of these $c\text{-}Kit^+$ fractions were very low in γ_c KO mice. Enforced expression of Bcl-2 significantly restores the numbers of $c\text{-}Kit^+$ precursors; 1.5-fold and 12-fold increases in $CD3^-c\text{-}Kit^+$ precursors were seen in Eμ-*bcl-2* γ_c KO and H2K-*bcl-2* γ_c KO mice, respectively. Among the $c\text{-}Kit^+$ populations, the transition of $CD3^-CD25^+c\text{-}Kit^{hi}$ (fraction II) to $CD3^-CD25^+c\text{-}Kit^{lo}$ (fraction III) was severely suppressed in the γ_c KO mice, suggesting that γ_c-mediated signals (probably IL-7R–mediated signals) might be critical for the expansion of thymocytes at these stages. Both *bcl-2* transgenes significantly restored these thymic precursors (Figure 3B). These data indicate that Bcl-2 rescues the expansion of thymic precursors as well as thymic positive selection.

Enforced Expression of Bcl-2 Does Not Restore Bone Marrow B Cell Precursors in γ_c-Deficient Mice

As shown in Figures 1 and 2A, $B220^+$ cells in blood remained scarce in both Eμ-*bcl-2* γ_c KO and H2K-*bcl-2* γ_c KO mice, but the increases in absolute numbers of B cells in these mouse strains were statistically significant compared to γ_c KO mice, suggesting that the enforced expression of Bcl-2 may result in accumulation of the rare mature B cells, as previously reported (MacDonnell et al., 1989; Strasser et al., 1991a). To assess the effect of Bcl-2 on B cell development in γ_c KO mice more clearly, we analyzed the B cell precursor populations in the bone marrow in each mouse line.

Table 1 shows absolute numbers of various bone marrow B cell populations (Hardy et al., 1991). Mature bone marrow B cells ($B220^+IgM^+$) were missing in γ_c KO mice irrespective of the presence of *bcl-2* transgenes (Figure

Table 1. Absolute Numbers of Cells in Thymus and Bone Marrow Subpopulations

	Thymus ($\times 10^5$)			Bone Marrow ($\times 10^5$)			
Mouse	Total	$CD69^+$	$CD3^-c\text{-}Kit^+$	Total	$B220^+IgM^+$	$B220^+IgM^-CD43^-$	$B220^+IgM^-CD43^+$
γ_c KO	68 ± 14	5.5 ± 1.5	0.098 ± 0.053	420 ± 76	0.19 ± 0.13	1.0 ± 0.74	8.8 ± 2.8
Eμ-*bcl-2* γ_c KO	280 ± 120[a]	37 ± 2.1[a]	0.14 ± 0.023	480 ± 73	0.20 ± 0.084	0.88 ± 0.45	9.0 ± 1.5
H2K-*bcl-2* γ_c KO	410 ± 120[a]	54 ± 12[a]	1.2 ± 0.52[a]	360 ± 88	0.21 ± 0.11	0.96 ± 0.77	6.7 ± 1.5
γ_c^+ (wild type)	2100 ± 380	270 ± 40	6.9 ± 1.8	560 ± 82	66 ± 13	110 ± 30	35 ± 12

Numbers of cells in bone marrow harvested from bilateral tibiae and femurs. Results are shown as means ± SD from 3–5 mice.

[a] Significant increases in numbers of thymocyte subpopulations in *bcl-2* transgenic γ_c KO mice as compared to those in γ_c KO mice ($p < 0.05$ by Student's t tests).

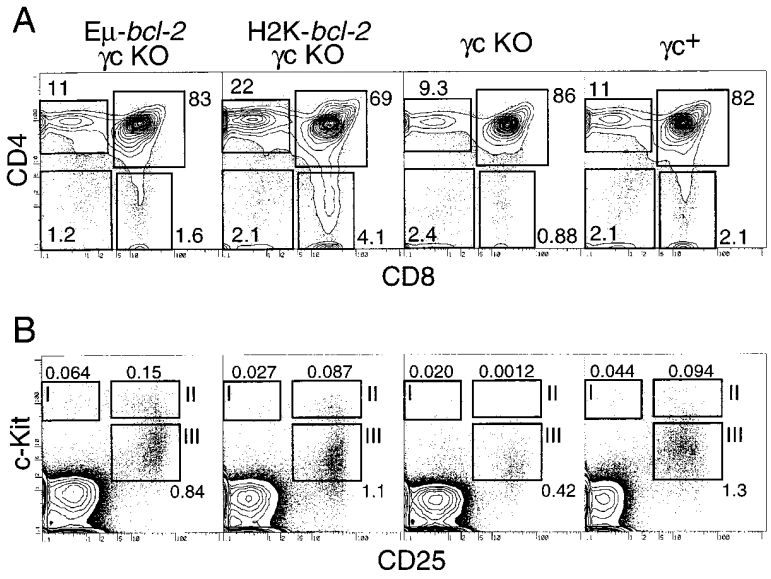

Figure 3. Enforced Expression of Bcl-2 Restores Development of Thymocytes in γ_c KO Mice

(A) CD4/CD8 profiles of total thymocytes in each mouse strain. Percentages of each subset (of total thymocytes) are also shown.

(B) c-Kit/CD25 profiles of $CD3^-$ thymocyte populations. Percentages of each fraction (I–III) in $CD3^-$ thymocytes are indicated.

4A). We subdivided the $B220^+IgM^-$ B cell precursors into two populations based on the expression of CD43 (Figure 4B). The $B220^+IgM^-CD43^+$ cells are reported to be pro-B cells that are rearranging the μ heavy chain genes, and $B220^+IgM^-CD43^-$ cells are pre-B cells that have completed rearrangement of the μ heavy chain genes and are rearranging the light chain genes (Hardy et al., 1991). H2K-*bcl-2* γ_c KO mice expressed Bcl-2 in virtually all immature $B220^+IgM^-$ cells, while the Eμ-*bcl-2* transgene was expressed in 15%–25% of immature $B220^+IgM^-$ cells (Figure 4C). The *bcl-2* transgenes did not restore the pro-B and pre-B cell populations; as shown in Figure 4B, the transition from $B220^+IgM^-CD43^+$ pro-B to $B220^+$ IgM^-CD43^- pre-B stages remained impaired, and absolute numbers of both populations remained low in both γ_c KO lines with *bcl-2* transgenes (Table 1). Accordingly, the *bcl-2* transgene did not affect the distribution and numbers of B cell precursors and mature B cells in the bone marrow, indicating that the receptor complexes, including γ_c, mediate developmental rather than survival signals in B lymphopoiesis.

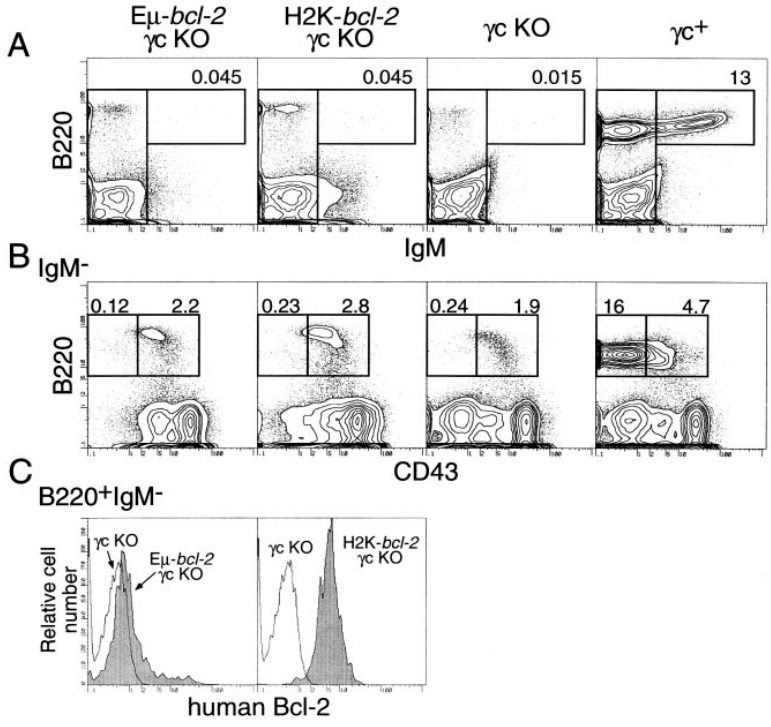

Figure 4. Enforced Expression of Bcl-2 Cannot Rescue B Cell Development in γ_c KO Mice

Flow cytometric analysis of bone marrow B cell precursors in each mouse strain.

(A) IgM/B220 profiles of total bone marrow cells. γ_c KO mice lack mature $B220^+IgM^+$ cells irrespective of the introduction of *bcl-2* transgenes.

(B) CD43/B220 profiles of IgM^- population. The transition from the $IgM^-B220^+CD43^+$ pro-B to the $IgM^-B220^+CD43^-$ pre-B stage was apparently impaired in γ_c KO strains. The numbers above the inset squares are the percentages of cells within the gate (of total bone marrow cells).

(C) *bcl-2* transgene expression in $B220^+IgM^-$ B cell precursors in bone marrow of each *bcl-2* transgenic γ_c KO strain (shaded histograms). Open histograms represent those of γ_c KO mice as negative controls. Virtually all B cell precursors in H2K-*bcl-2* γ_c KO mice expressed human Bcl-2.

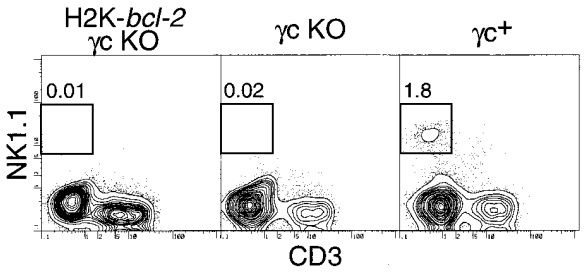

Figure 5. Enforced Expression of Bcl-2 Cannot Rescue NK Cell Development in γ_c KO Mice

Flow cytometric analysis of nucleated cells in the spleen. Numbers accompanying inset boxes indicate the percentage of the gated population in splenocytes. NK1.1$^+$CD3$^-$ NK cells could not be detected in either H2K-*bcl-2* γ_c KO or γ_c KO mice.

Enforced Expression of Bcl-2 Does Not Restore NK Cells in γ_c-Deficient Mice

We could not detect a significant number of NK cells in either blood or spleens of γ_c KO mice, as reported previously (Cao et al., 1995; DiSanto et al., 1995; Ohbo et al., 1996). Neither the H2K-*bcl-2* nor the Eμ-*bcl-2* transgene could rescue NK cell development in the γ_c KO mice; as shown in Figure 5, NK1.1$^+$CD3$^-$ cells were absent from the spleens of H2K-*bcl-2* γ_c KO mice, although the H2K-*bcl-2* transgene is expressed in the NK1.1$^+$CD3$^-$ NK cells (J. D. and I. L. W., unpublished data).

Discussion

Interaction between cytokines and cytokine receptors appears to signal cells to proliferate, to induce the expression of lineage-associated genes (differentiation), and to maintain their survival (Watowich et al., 1996). These various roles of cytokines may be ascribed to the activation of independent signaling pathways initiated from the different functional domains of the signaling subunits of cytokine receptors (Fukunaga et al., 1993; Kinoshita et al., 1995; Fukada et al., 1996). A significant role for some cytokines seems to be to prevent cells from undergoing apoptosis, rather than to direct cell differentiation and/or proliferation; various cytokine-dependent cell lines that undergo apoptotic cell death after cytokine deprivation survive and complete their differentiation program in vitro after introduction of survival genes such as *bcl-2* (Fairbairn et al., 1993; Cory, 1995; Yang and Korsmeyer, 1996).

We have recently shown that Bcl-2 can substitute for signals induced by cytokines such as IL-7 and macrophage colony- stimulating factor (M-CSF) in vivo; introduction of the Eμ-*bcl-2* transgene in IL-7Rα KO mice resulted in rescue of T lymphopoiesis (Akashi et al., 1997), and introduction of the *bcl-2* transgene into myelomonocytic cells resulted in significant recovery of macrophage development and bone remodeling in osteopetrotic M-CSF–deficient mice (*op/op* mice) (Lagasse and Weissman, 1997). In addition to our reports, others also have reported the rescue of T lymphocyte development by introducing the Eμ-*bcl-2*-38 transgene (Strasser et al., 1991b) into IL-7R–deficient mice (Maraskovsky et al., 1997). Although it remains possible that Bcl-2 plays a role in activating intracellular maturation programs in these experiments, these data strongly suggest that cell survival signals are the principal function of certain cytokines, including IL-7 and M-CSF.

In this study, the *bcl-2* transgenes significantly restored T cells, but not B or NK cells, in a γ_c KO mouse that is a mouse model of X-SCID in humans. The restoration of T cells in these *bcl-2* transgenic γ_c KO mice could be ascribed to the rescue of thymocytes undergoing positive selection. Since Bcl-2 itself does not support thymic positive selection in the absence of MHC (Linette et al., 1994; Tao et al., 1994; Akashi et al., 1997), this study shows that Bcl-2 can substitute for signals from γ_c-related cytokine receptors during positive selection. Together with the recovery of T lymphopoiesis in Eμ-*bcl-2* IL-7Rα KO mice (Akashi et al., 1997), it is strongly suggested that Bcl-2 substitutes mainly for IL-7R–mediated survival signals so that they can complete their developmental programs as directed by positive selection signals from T cell receptor–MHC interaction.

The γ_c-related cytokines (mainly IL-7) might also play an important role at the early stage of thymic development; the restoration of T lineage cells was more profound in H2K-*bcl-2* γ_c KO mice than in Eμ-*bcl-2* γ_c KO mice. This might be because the H-2K promotor efficiently drives *bcl-2* transgene expression in all stages of T cell development, including the earliest thymic precursors. Since the proliferation of CD3$^-$CD25$^+$c-Kit$^+$ thymic precursors are dependent on Slf as well as IL-7 (Suda and Zlotnik, 1991; Rodewalt et al., 1995), the enforced expression of Bcl-2 might substitute for IL-7–mediated survival signals that would allow them to proliferate in the presence of c-Kit– and pre-T cell receptor complex–mediated signals (Fehling et al., 1995; Akashi et al., 1997). However, it is possible that IL-7 may in part play a role in the proliferation of thymocytes, because the restoration is significant but not complete even in H2K-*bcl-2* γ_c KO mice.

On the other hand, the enforced expression of Bcl-2 could restore neither mature B cells nor B cell precursors in γ_c KO mice. The slight increase in blood B220$^+$ cells might be the result of prolonged cell survival, because expression of Bcl-2 promotes accumulation of B cells (MacDonnell et al., 1989; Strasser et al., 1991a). Among γ_c-related cytokines, IL-7 has been shown to induce nonredundant signals in B as well as T cell development (Peschon et al., 1994; von Freeden-Jeffry et al., 1995). This nonredundancy may be attributable to the critical role of IL-7 on two stages of B cell development: it has been shown that IL-7 promotes rearrangement of the μ heavy chain gene of pro-B cells at least in vitro (Corcoran et al., 1996) and stimulates proliferation of pre-B cells (Reichman-Fried et al., 1993; Spanopoulou et al., 1994; Young et al., 1994). These two events might be closely related because during the transition from the pro-B to the pre-B stage, the formation of the pre-B receptor complex that is composed of a μ chain and surrogate light chains (λ5 and VpreB) is indispensable for proliferation (Karasuyama et al., 1996). The impairment of the

transition from pro-B to pre-B in γ_c KO mice suggests an important role for IL-7 (or other γ_c-related cytokines) in μ heavy chain gene rearrangement or in formation of the pre-B receptor complex in vivo. Alternatively, since IL-7 plays a role in the proliferation of cells on the transition from the pro-B to the pre-B stage (Karasuyama et al., 1996), this phenotype may result simply from impairment of proliferation of these cells. IL-7 signaling also plays a role in differentiation of pre-B cells, since the cell surface aminopeptidase BP-1/6C3 expression in pro-B cells can be induced by IL-7 (Sherwood and Weissman, 1990). This study shows that Bcl-2 cannot substitute for IL-7R signaling in these processes of B cell development.

Although common lymphoid progenitors in bone marrow (M. K. et al., unpublished data) as well as the earliest thymic precursors (Rodewald et al., 1992; Matsuzaki et al., 1993) have been shown to give rise to NK cells in vivo, the precise maturation pathway of NK cells has not been clarified. γ_c KO mice have been shown to lack NK cells (Cao et al., 1995; DiSanto et al., 1995; Ohbo et al., 1996). This deficiency may be due to the lack of formation of functional IL-15R in the mice (Giri et al., 1994; Suzuki et al., 1997). The critical stage of NK cell development that is supported by γ_c-mediated signals (including IL-15R–mediated signals) is still unclear. As in the case of B lineage cells, γ_c-mediated signals may be necessary to direct the differentiation program of NK cell maturation or may be important for expanding NK cell precursor populations at certain maturational stages.

Thus, this study suggests that in addition to survival signals in T cell development, γ_c-related cytokines induce a variety of signals in the development of lymphoid cells. Janus kinase 1 (Jak1) and Jak3 tyrosine kinases are commonly used as triggering molecules in signal transduction pathways initiating from γ_c-related receptor complexes, including IL-7R and IL-15R (Ihle, 1995). Since Bcl-2 could substitute for nonredundant signals from IL-7R in T lymphopoiesis but not in B lymphopoiesis, the results strongly suggest that intracytoplasmic signaling cascades activated by heterodimerization of IL-7Rα and γ_c might be different for T and B cell maturation. Future studies of the signal transduction pathways responsible for the proliferation, differentiation, and survival of these lymphoid cells will be required to clarify the precise role of γ_c-related cytokines in lymphopoiesis.

Experimental Procedures

Mice

Eμ-*bcl-2*-25 transgenic mice, in which the human *bcl-2* transgene is expressed mainly in T lineage cells (Strasser et al., 1991b), were provided by S. Cory (The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia). The H2K-*bcl-2* transgenic mouse (line 1043) was generated with a construct composed of H-2Kb promoter (HindIII–NruI) located upstream of the cDNA for human Bcl-2 and the MoMuLV long terminal repeat sequence at the 3′ untranslated region of the cDNA to enhance its expression (pH2K-BCL-2) (J. D. and I. L. W., unpublished data). The γ_c KO mice were established as described previously (Ohbo et al., 1996). All mice were on the C57BL/6 or C57BL/Ka-Thy1.1 background (backcrossed more than three generations) and bred in the animal care facility at Stanford University School of Medicine. All mice were analyzed at 4–5 weeks of age.

Antibodies

Antibodies used for flow cytometric analysis were as follows. Fluorescein isothiocyanate (FITC)– or phycoerythrin (PE)–conjugated anti-CD3 (KT31.3), allophycocyanin (APC)–conjugated anti-c-Kit (2B8), PE- or APC-conjugated anti-B220 (RA-6B2), FITC-conjugated anti-Mac-1 (M1/70), FITC-conjugated anti-Gr-1 (RB6–8C5), and FITC-conjugated anti-IL-2Rα (PC61) were affinity purified from culture supernatant from hybridoma cells with protein G column chromatography (protein G–Sepharose, Pharmacia, Uppsala, Sweden) and labeled in our laboratory. FITC- or PE-labeled anti-CD43 (S7) and FITC-labeled anti-CD69 (H1.2F3), Texas Red-labeled anti-IgM (affinity purified rabbit polyclonal antibody), and FITC-labeled anti-human Bcl-2 (clone 124), which were purchased from Pharmingen (San Diego, CA), Caltag (Burlingame, CA), and DAKO (Carpinteria, CA), respectively. PE-labeled anti-IgM (clone 331) was obtained from L. A. Herzenberg (Stanford University School of Medicine).

Preparation of Cell Suspensions

Cells were obtained from thymi and spleens that were crushed between glass slides and resuspended in staining medium (Hank's balanced salt solution with 2% fetal calf serum and 0.02% NaN_3). Cell suspensions from bone marrow were obtained by flushing femurs and tibiae with the staining medium. Cells were treated with ammonium chloride–potassium bicarbonate (ACK) solution (150 mM NH_4Cl and 10 mM $KHCO_3$) to lyse red blood cells. Cell debris were depleted by passage through nylon mesh screens, and cells were counted with a Coulter Counter model ZM (Coulter, Miami, FL). Blood samples were collected from tail veins into 10 μl of 0.1 M EDTA in phosphate-buffered saline in microcentrifuge tubes. Nucleated blood cells were counted with a Coulter Counter after treatment with Zap-Oglobin II (Coulter). For antibody stainings, nucleated cells were enriched by Dextran segmentation using 0.5% Dextran in phosphate-buffered saline and treated with ACK.

Flow Cytometric Analysis

Cells (1×10^6) were stained in 50 μl of staining medium with fluorochrome-conjugated antibodies on ice for 20 min. After the cells were washed with staining medium, they were resuspended in staining medium with propidium iodide. Intracytoplasmic human Bcl-2 staining was performed as described previously (Akashi et al., 1997); the cells were suspended in 50 μl of staining media containing 0.03% saponin and FITC-conjugated anti-human Bcl-2 for 30 min. Cells were analyzed on a highly modified FACS II (Beckton Dickinson, Mountain View, CA) with dual lasers (488 nm argon laser and 599 nm dye laser) or FACScan (Beckton Dickinson) at the Stanford shared FACS facility. Fluorescence data were analyzed by the FACS/DESK program and presented as two-parameter probability plots (5%) or histograms.

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